# FavorPrep™
# Blood / Cultrued Cell
# Total RNA Purification Mini Kit

## User Manual

**Cat. No.: FABRK 001 (50 Preps)**
**FABRK 001-1 (100 Preps)**
**FABRK 001-2 (300 Preps)**

**For Research Use Only**

v.1304

## Introduction

FavorPrep Blood/ Cultured Cell Total RNA Extraction Mini Kit is desigened for extraction of total RNA from whole blood and cultured cells. Some specially modified protocols are developed for other samples, such as bacteria and yeast. This method first lyses cells by using a chaotropic salt , then binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH2O. It takes 30 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library contruction.

## Sample amount and yield:

Sample: Up to 0.3 ml fresh whole blood
Up to 1 X $10^7$ animal cultured cells
Up to 1 X $10^9$ bacterial cells
Up to 5 X $10^7$ yeast

Handling time: about 30 min

## Kit Contents

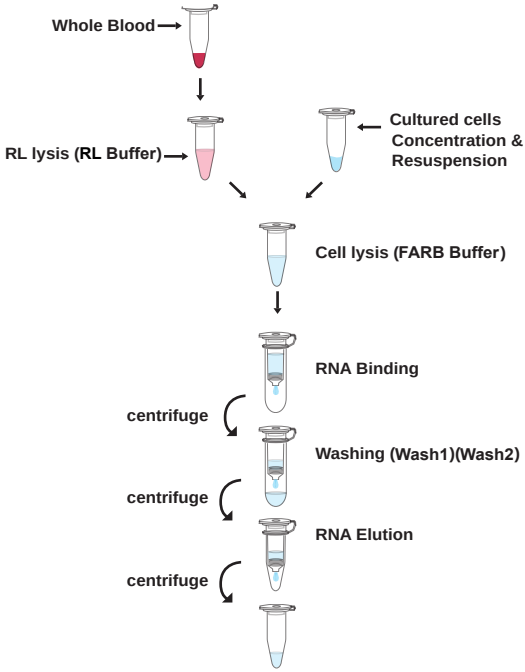
| Cat. No. / preps | FABRK001 (50 preps) | FABRK001-1 (100 preps) | FABRK001-2 (300 preps) |
|---|---|---|---|
| RL Buffer | 120 ml | 240 ml | 240 ml X 3 |
| FARB Buffer | 25 ml | 45 ml | 130 ml |
| Wash Buffer 1 | 30 ml | 60 ml | 170 ml |
| Wash Buffer 2 (concentrated) | 15 ml * | 35 ml ** | 50 ml *** x 2 |
| RNase-free ddH2O | 6 ml | 6 ml | 8 ml X2 |
| Filter Column | 50 pcs | 100 pcs | 300 pcs |
| FARB Mini Column | 50 pcs | 100 pcs | 300 pcs |
| Collection Tube | 100 pcs | 200 pcs | 600 pcs |
| Elution Tube | 50 pcs | 100 pcs | 300 pcs |
| User manual | 1 | 1 | 1 |

* Add 60 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
** Add 140 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
*** Add 200 ml ethanol (96-100 %) to each Wash Buffer 2 when first open.

## Important notes

1. Make sure the starting sample amount is under the limit.
2. Make sure everything is RNase-free when handling RNA.
3. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling hese buffers.
4. Pipet a required volume of FARB Buffer to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-ME) per 1ml FARB Buffer before use.
5. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 as bottle indicated when first open.
6. The additional equipment, 20-G needle syringe, is needed for extraction of total RNA from tissue sample.
7. Dilute RNase-free DNase 1 in reaction buffer to final conc. = 0.5 U/µl.

## Brief Procedure



## Genernal Protocol: (For Human Whole Blood)

**Please Read Important Notes Before Starting The Following Steps.**

1. **Collect fresh human blood in an anticoagulant-treat collection tube.**

2. **Add 200~300 µl human whole blood to an appropriately sized micro-centrifuge tube (1.5 ml or 2.0 ml tube). (not provided)**
   --If the sample volume is more than 200 ul, use a 2.0 ml tube as the sample container.

3. **Mix 5 volume of RL Buffer with 1 volume of the sample and mix well by inversion.**

4. **Incubate on ice for 10 min. Vortex briefly 2 times during incubation.**

5. **Centrifuge for 1 min at 4,500 rpm to form a cell pellet and discard the supernatant completely.**

6. **Add 600 µl of RL Buffer to resuspend the cell pellet by briefly vortexing.**

7. **Centrifuge for 1min at 4,500 rpm to form a cell pellet again and discard the supernatant completely.**

8. **Add 350 µl of FARB Buffer (ß-ME added) to the cell pellet and vortex vigoruusly. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), See Important Note: 3)

   **Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.**

9. **Transfer the sample mixture to Filter Column Set and centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min.**

10. **Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided), and adjust the volume of the clear lysate.**
    --Avoid to pipette any debris and pellet from the Collection Tube.

11. **Add 1 volume of 70% ethanol to the clear lysate and mix well by vortexing.**

12. **Transfer the ethanol added sample (including any precipitate) to FARB Mini Column Set. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min and discard the flow-through.**

**13.(Optional): To eliminate genomic DNA contamination, follow the steps from 13a. Otherwise, proceed to step14 directly.**

**13a. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**

**13b. Add 60 µl of RNase-free DNase 1 solution (0.5U/µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.**

**13c. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**

**13d. After DNase 1 treatment, proceed to step 15.**

**14. Add 500 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**

**15. Wash FARB Mini Column twice with 700 µl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**
--Make sure that ethanol has been added into Wash Buffer 2 when first open.

**16. Centrifuge at full speed (14,000 rpm or 10,000 x g) for an additional 3 min to dry the column.**
--**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

**17. Place FARB Mini Column to Elution Tube.**

**18. Add 50 µl of RNase-free Water to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.**
--**Important Step!** For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

**19. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to elute RNA.**

**20. Store RNA at -70°C.**

## Special Protocol: (For Animal Cells)

1. **Pellet 1 ~ 5 ×$10^6$ cells by centrifuge at 300 x g for 5 min. Remove all the supernatant.**
2. **Add 350 µl of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)

> **Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.**

3. **Transfer the mixture to Filter Column Set and centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min.**
4. **Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided) and adjust the volume of the clear lysate.**
   --Avoid pipetting any debris and pellet from this Collection Tube.
5. **Add 1 volume of 70% ethanol to the clear lysate and mix well by pipetting.**
6. **Follow the General Protocol starting from step 12.**

## Special Protocol: (For Bacteria)

1. **Transfer 1 ml well-grown bacterial culture (or up to 1x$10^9$ cells) to a microcentrifuge tube (not provided).**
2. **Descend the bacterial cells by centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min and discard the supernatant completely.**
3. **Resuspend the cell pellet in 100 µl RNase-free lysozyme reaction solution (20mg/ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition) (not provided).**
4. **Incubate at 37°C for 10 min.**
5. **Add 350 µl of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)

> **Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.**

6. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to spin down insouble material and transfer the supernatant to a microcentrifge tube. (not provided)**

7. **Add 1 volume of 70% ethanol to the clear lysate and mix by pipetting.**

8. **Follow the General Protocol starting from step 12.**

## Special Protocol: (For Yeast)

1. **Transfer 3 ml log-phase (OD600=10) yeast culture to a microcentrifuge tube. (not provided)**

2. **Descend the yeast cells by centrifug at 7,500 rpm (5,000 x g) for 10 min and discard the supernatant completely.**

3. **Resuspend the cell pellet in 600 µl sorbitol buffer (1M sorbitol; 100mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolase or lyticase and incubate at 30 °C for 30 min.**
   --Prepare sorbitol buffer just before use.

4. **Centrifuge at 7,500rpm (5,000xg) for 5 min. Remove the supernatant by pipetting.**

5. **Add 350µl of FARB Buffer (ß-ME added) to the sample and mix well by vortexing. Incubate at room temperature for 5 min.**

   **Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.**

6. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to spin down insoluble materials and transfer the supernatant to a microc-entrifuge tube (not provided).**

7. **Add 250 µl of ethanol (96-100%) to the clear lysate and mix by pipetting.**

8. **Follow the General Protocol starting from step 12.**

# FavorPrep™ Blood / Cultured Cell Total RNA Purification Maxi Kit

## User Manual

**Cat. No.: FABRK 003 (10 Preps)**
**FABRK 003-1 (24 Preps)**

**For Research Use Only**

v.1005

Australian distributors:
Fisher Biotec Australia
free call: 1800 066 077
email: info@fisherbiotec.com
web: www.fisherbiotec.com

## Introduction

FavorPrep Blood/ Cultured Cell Total RNA Extraction Maxi Kit is desigened for extraction of total RNA from whole blood and cultured cells. Some specially modified protocols are developed for other samples, such as bacteria and yeast. This method first lyses cells by using a chaotropic salt , then binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free Water. It takes 60 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library contruction.

## Sample amount:

3~10 ml of human whole blood

Up to 5 X $10^{10}$ bacteria cells

Up to 5 X $10^{9}$ yeast culture

Up to 5 ×$10^{8}$ of animal cells

Handling time: about 60 min

## Kit Contents

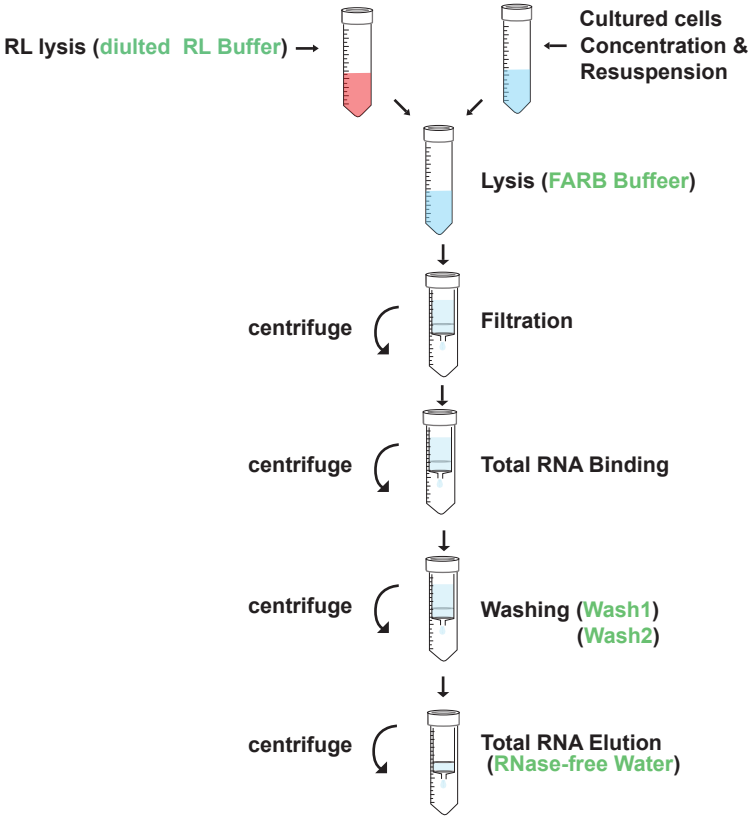
| Cat. No. / preps | FABRK003 (10 preps) | FABRK003 (24 preps) |
|---|---|---|
| 10 X RL Buffer | 100 ml | 100 ml x 2 |
| FARB Buffer | 150 ml | 180 ml x 2 |
| Wash Buffer 1 | 135 ml | 160 ml x 2 |
| Wash Buffer 2 (concentrated) | 27 ml x 2 | 27 ml x 5 |
| RNase-free Water | 12 ml | 30 ml |
| Filter Column | 10 pcs | 24 pcs |
| FARB Maxi Column | 10 pcs | 24 pcs |
| Elution Tube (50 ml tube) | 10 pcs | 24 pcs |
| User manual | 1 | 1 |

* Add 108 ml ethanol (96-100 %) to each Wash Buffer 2 when first open.

## Important notes

1. Make sure everything is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling hese buffers.
3. Pipet a required volume of FARB Buffer to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-ME) per 1ml FARB Buffer before use.
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 as bottle indicated when first open.
5. Dilute RNase-free DNase 1 in reaction buffer (150mM NaCl, 1 mM $MgCl_2$, 10 mM Tris HCl, pH 7.5) to final conc. = 2KU/ml. (1 ml /preparation)
6. Use a centrifuge with a swinging bucket rotor for 15ml (Midi) or 50ml (Maxi) in all centrifugation steps. The maximum speed should be 3500-5000 rpm or 3000-5000 x g.

## Brief Procedure



## Genernal Protocol: (For Human Whole Blood)

**Please Read Important Notes Before Starting The Following Steps.**

1. **Collect fresh human blood in an anticoagulant-treat collection tube.**

2. **Add 3 ~10 ml of human whole blood to an appropriately sized centrifuge tube (15 ml or 50 ml tube). (not provided)**

3. **Mix 5 volume of diluted RL Buffer with 1 volume of the sample and mix well by inversion.**
   **Note:** RL buffer is provided as a 10 x concentrate which would must be diluted with sterile deionized water before use.
   For example, add 25 ml of diluted RL Buffer to 5 ml of blood sample.

4. **Incubate at room temperature for 5 min. Vortex briefly 2 times during incubation.**

5. **Centrifuge for 5 min at 500 x g to form a cell pellet and discard the supernatant completely.**

6. **Add 2 volume of diluted RL Buffer to wash the cell pellet by briefly vortexing.**

7. **Centrifuge for 5 min at 500 x g to form a cell pellet again and discard the supernatant completely.**

8. **Add 12.5 ml of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 3 min to lyse cells completely.**
   (For preparation of FARB Buffer (ß-ME added), See Important Note: 3)

> **Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.**

9. **Place a Filter Maxi Column into a clean 50 ml tube (not provided), and transfer the sample mixture to Filter Column, centrifuge at full speed for 5 min.**

10. **Transfer the clarified supernatant from previous step to a clean 50 ml tube (not provided), and adjust the volume of the clear lysate.**
    --Avoid to disrupt any debris and pellet when transfer the supernatant.

11. **Add an equal volume of 70% ethanol to the clear lysate and mix well by vortexing.**

**12. Place a FARB Maxi Column in a clean 50 ml tube (not provided), and transfer 14 ml of the ethanol added sample (including any precipitate) to FARB Maxi Colum, centrifuge at full speed for 5 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**
---The maximum capacity of FARB Midi Column is 14 ml, repeat Step 7 for the remaining sample mixture.

**13.(Optional): To eliminate genomic DNA contamination, follow the steps from 13a. Otherwise, proceed to step14 directly.**

**13a. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column. Centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**

**13b. Add 1 ml of RNase-free DNase 1 solution (2U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 10 min.**

**13c. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column. Centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**

**13d. After DNase 1 treatment, proceed to step 15.**

**14. Add 12.5 ml of Wash Buffer 1 to wash FARB Maxi Column. Centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**

**15. Wash FARB Maxi Column twice with 12.5 ml of Wash Buffer 2 by Centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**
--Make sure that ethanol has been added into Wash Buffer 2 when first open.

**16. Centrifuge at full speed (>4,000 x g) for an additional 10 min to dry the FARB Maxi column.**
--**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

**17. Place FARB Maxi Column to Elution Tube (50 ml tube, provided).**

**18. Add 500 ~1000 µl of RNase-free Water to the membrane center of FARB Maxi Column. Stand FARB Maxi Column for 5 min.**
--**Important Step!** For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

**19. Centrifuge at full speed for 5 min to elute RNA.**

**20. Store RNA at -70°C.**

## Special Protocol: (For Animal Cells)

1. **Pellet Up to 5 ×$10^8$ of animal cells by centrifuge at 300 x g for 5 min. Discard the supernatant completely.**
2. **Add 14 ml of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
3. **Place a Filter Maxi Column in a 50 ml tube (not provided), and transfer the sample mixture to Filter Maxi Column, centrifuge at full speed for 5 min.**
4. **Transfer the clarified supernatant from previous step to a clean 50 ml tube (not provided) and adjust the volume of the clear lysate.**
   --Avoid pipetting any debris and pellet from this Collection Tube.
5. **Add an equal volume of 70% ethanol to the clear lysate and mix well by pipetting.**
6. **Follow the Genernal Protocol starting from step 12.**

## Special Protocol: (For Bacteria)

1. **Transfer Up to 5 X $10^{10}$ of well-grown bacterial to a centrifuge tube (not provided).**
2. **Descend the bacterial cells by centrifuge at >3,000 x g for 5 min and discard the supernatant completely.**
3. **Resuspend the cell pellet in 1 ml of RNase-free lysozyme reaction solution (20mg/ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition) (not provided).**
4. **Incubate at 37°C for 10 min.**
5. **Add 13 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
6. **Centrifuge at full speed for 5 min to spin down insouble material and transfer the supernatant to a 50 ml tube. (not provided)**
7. **Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.**
8. **Follow the Genernal Protocol starting from step 12.**

## Special Protocol: (For Yeast)

1. **Transfer up to 5 X $10^9$ yeast cells to a 50 ml centrifuge tube. (not provided)**

2. **Descend the yeast cells by centrifug at 500 x g at 4 °C for 5 min and discard the supernatant completely.**

3. **Resuspend the cell pellet in 2.5 ml of enzymatic lysis buffer (20 mg/ml lyticase or zymolase; 1M sorbitol; 100mM EDTA; 0.1% ß-ME) (not provided). And incubate at 30 °C for 30 min.**
   --Prepare sorbitol buffer just before use.

4. **Centrifuge at 500 x g at room temperature for 5 min to pellet spheroplasts and discard the supernatant completely.**

5. **Add 14 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortexing. Incubate at room temperature for 5 minutes.**

6. **Centrifuge at full speed for 5 min to spin down insoluble materials and transfer the clarified supernatant to a 50 ml tube (not provided).**

7. **Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.**

8. **Follow the Genernal Protocol starting from step 12.**